

PATENT

What is claimed:

1. An animal cell which can be infected by influenza viruses and is adapted to growth in suspension, wherein said cell is of the cell line MDCK (ATCC CCL 34 MDCL (NBL-2)).
2. The animal cell of claim 1, wherein said cell is of the cell line MDCK 33016 (DSM ACC 2219).
3. An animal cell which can be infected by influenza viruses and is adapted to growth in serum-free medium, wherein said cell is of the cell line MDCK (ATCC CCL 34 MDCK (CCL-2)).
4. The animal cell of claim 3, wherein said cell is of the cell line MDCK 33016 (DSM ACC 2219).
5. A process for the replication of influenza viruses in cell culture, which comprises:
 - (i) proliferating cells of the cell line MDCK (ATCC CCL MDCK (NBL-2)) which can be infected by influenza viruses in serum-free medium;
 - (ii) infecting the cells with influenza viruses;
 - (iii) shortly before infection, simultaneously with infection or shortly after infection, adding to the cell suspension a protease to cleave the precursor protein of hemagglutinin; and
 - (iv) after a further culturing phase, isolating the influenza viruses replicated in the cells.

PATENT

6. The process as claimed in claim 5, the culture of the cells taking place in the perfusion system.

7. The process as claimed in claim 5, the culture of the cells taking place in the batch process.

8. The process as claimed in claim 5, the pH of the culture medium in step (i) being in the range from 6.6 to 7.8.

9. The process as claimed in claim 8, the pH of the culture medium being in the range from 6.8 to 7.3.

10. The process as claimed in claim 5, the infection with influenza viruses being carried out when the cell culture has achieved a cell density of about $8 \text{ to } 25 \times 10^5$ cells/ml (batch process) or of about $5 \text{ to } 20 \times 10^6$ cells/ml (perfusion process).

11. The process as claimed in claim 5, the infection of the cells with influenza viruses being carried out at an m.o.i. (multiplicity of infection) of about 0.001 to 10.

12. The process as claimed in claim 11, the infection being carried out at an m.o.i. of about 0.002 to 0.5.

PATENT

13. The process as claimed in claim 5, the protease being a serine protease.
14. The process as claimed in claim 13, the serine protease being trypsin.
15. The process as claimed in claim 14, trypsin being added up to a final concentration in the culture medium of 1 to 200 $\mu\text{g/ml}$.
16. The process as claimed in claim 15, the final concentration of trypsin in the culture medium being in the range from 5 to 50 $\mu\text{g/ml}$.
17. The process as claimed in claim 5, the infected cells being cultured for 2 to 10 days.
18. The process as claimed in claim 17, the infected cells being cultured for 3 to 7 days.
19. The process as claimed in claim 5, the infected cells being cultured at 30 °C to 36 °C.
20. The process as claimed in claim 19, the infected cells being cultured at 32 °C to 34 °C.
21. The process as claimed in claim 5, the harvesting and isolation of the replicated viruses being carried out 2 to 10 days after infection.
22. The process as claimed in claim 21, the harvesting and isolation of the viruses being

carried out 3 to 7 days after infection.

23. The process as claimed in claim 5, wherein the cells are of the cell line MDCK 33016 (DSM ACC 2219).

24. A process for the replication of influenza viruses in cell culture, which comprises:

- (i) proliferating cells of the cell line MDCK (ATCC CCL MDCK (NBL-2)) which can be infected by influenza viruses in suspension;
- (ii) infecting the cells with influenza viruses;
- (iii) shortly before infection, simultaneously with infection or shortly after infection, adding to the cell suspension a protease to cleave the precursor protein of hemagglutinin; and
- (iv) after a further culturing phase, isolating the influenza viruses replicated in the cells.

25. The process as claimed in claim 24, the culture of the cells taking place in the perfusion system.

26. The process as claimed in claim 24, the culture of the cells taking place in the batch process.

27. The process as claimed in claim 24, the pH of the culture medium in step (i) being in the range from 6.6 to 7.8.

PATENT

28. The process as claimed in claim 27, the pH of the culture medium being in the range from 6.8 to 7.3.

29. The process as claimed in claim 24, the infection with influenza viruses being carried out when the cell culture has achieved a cell density of about 8 to 25×10^5 cells/ml (batch process) or of about 5 to 20×10^6 cells/ml (perfusion process).

30. The process as claimed in claim 22, the infection of the cells with influenza viruses being carried out at an m.o.i. (multiplicity of infection) of about 0.001 to 10.

31. The process as claimed in claim 30, the infection being carried out at an m.o.i. of about 0.002 to 0.5.

32. The process as claimed in claim 24, the protease being a serine protease.

33. The process as claimed in claim 32, the serine protease being trypsin.

34. The process as claimed in claim 33, trypsin being added up to a final concentration in the culture medium of 1 to 200 $\mu\text{g/ml}$.

35. The process as claimed in claim 34, the final concentration of trypsin in the culture medium being in the range from 5 to 50 $\mu\text{g/ml}$.

PATENT

36. The process as claimed in claim 24, the infected cells being cultured for 2 to 10 days.
37. The process as claimed in claim 36, the infected cells being cultured for 3 to 7 days.
38. The process as claimed in claim 24, the infected cells being cultured at 30 °C to 36 °C.
39. The process as claimed in claim 38, the infected cells being cultured at 32 °C to 34 °C.
40. The process as claimed in claim 24, the harvesting and isolation of the replicated viruses being carried out 2 to 10 days after infection.
41. The process as claimed in claim 40, the harvesting and isolation of the viruses being carried out 3 to 7 days after infection.
42. The process as claimed in claim 24, wherein the cells are of the cell line MDCK 33016 (DSM ACC 2219).